Supplementary figures related to

Systematic identification of silencers in human cells

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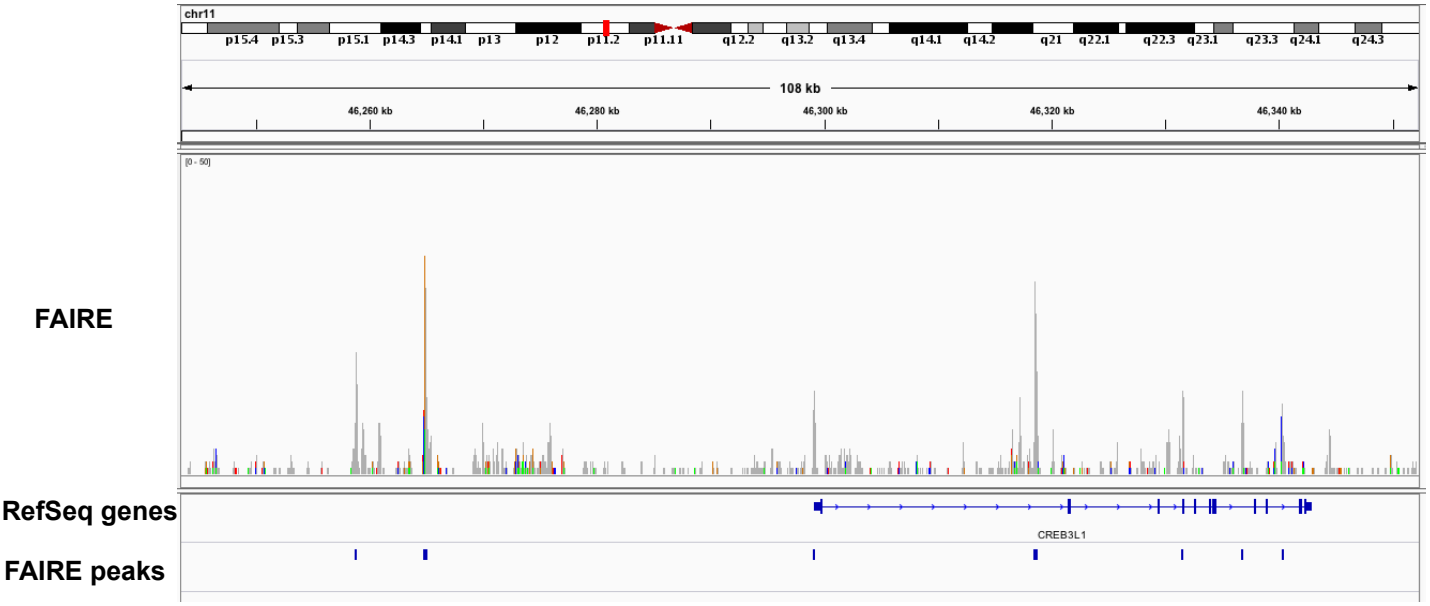
a



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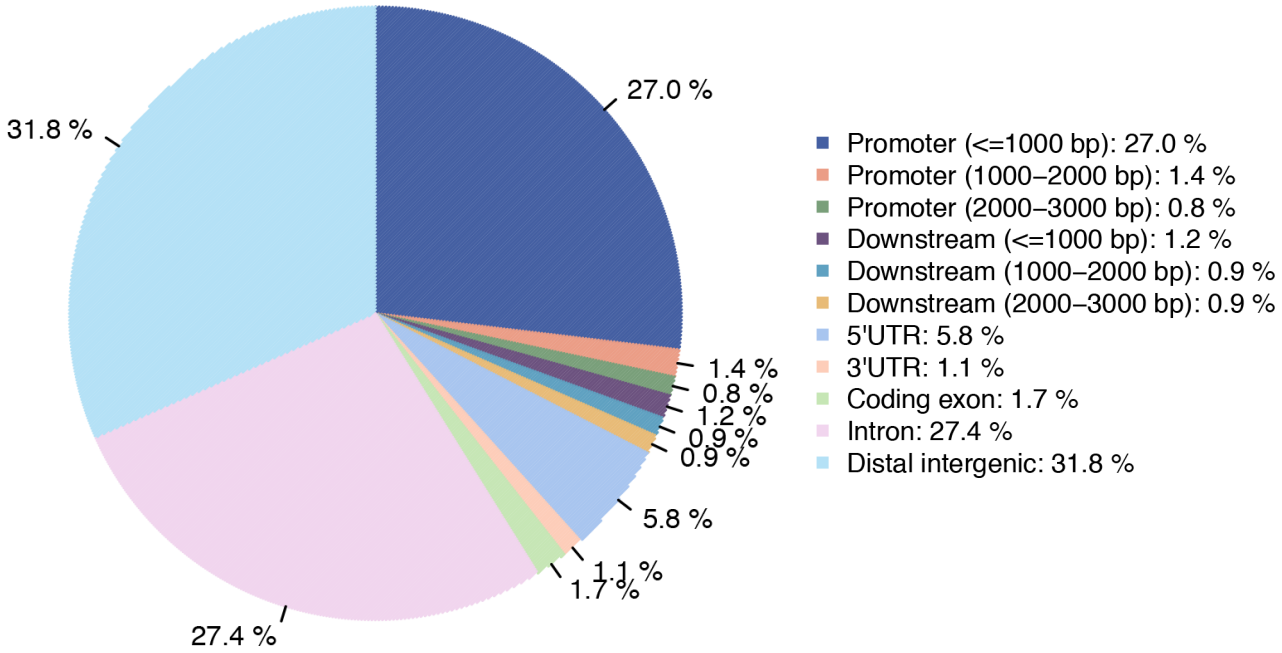
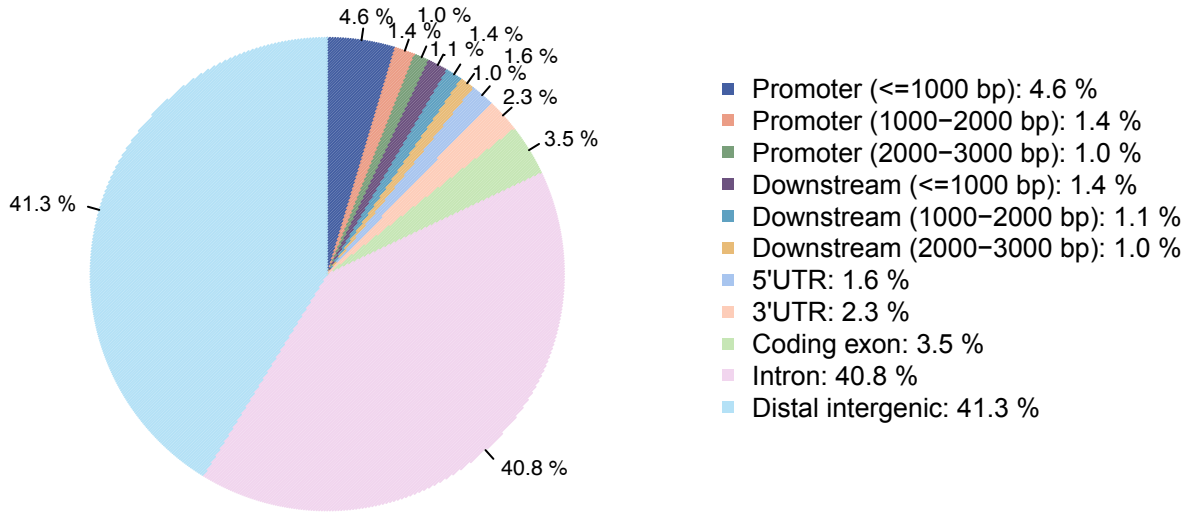
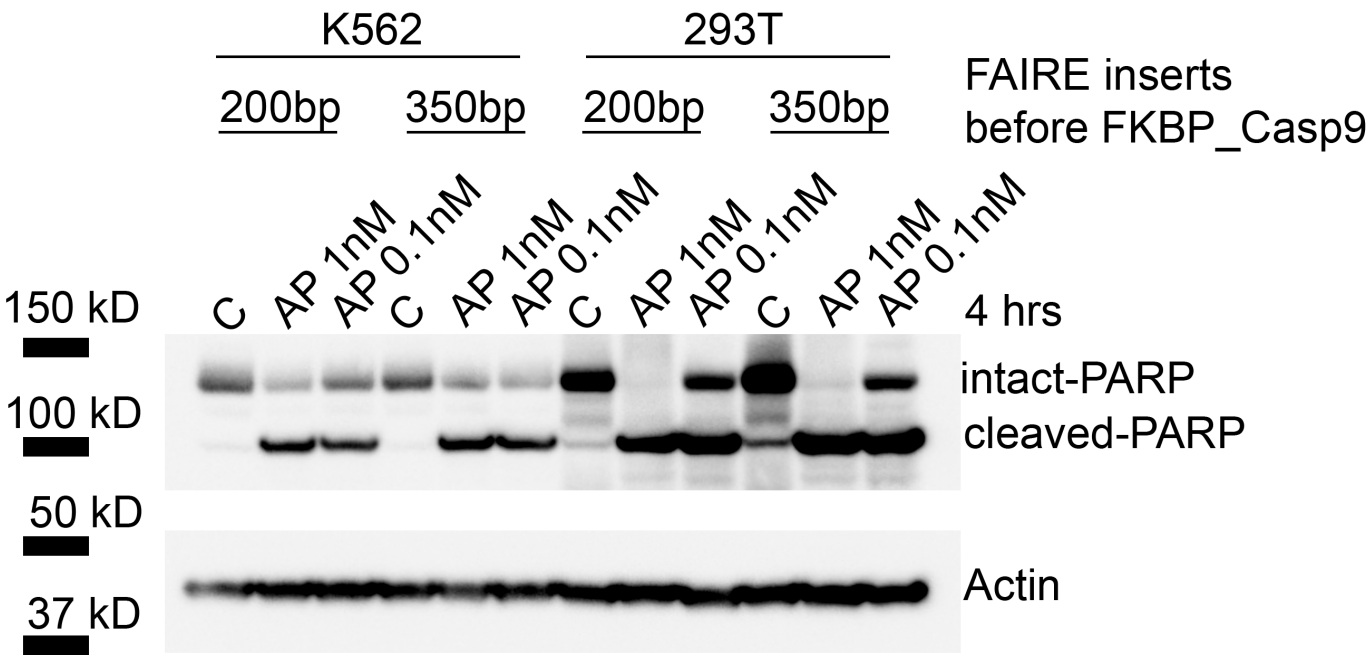


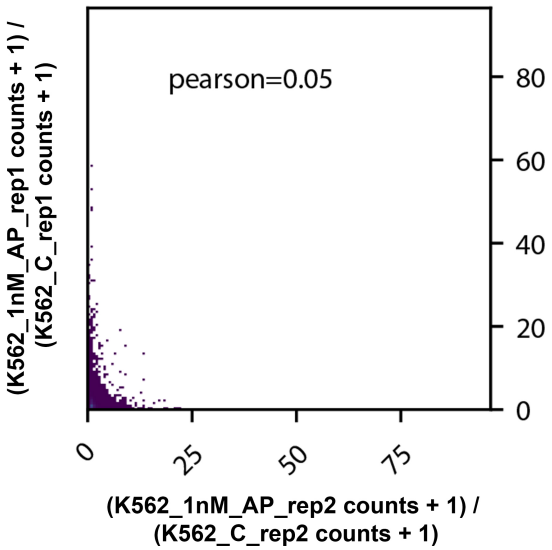
Supplementary Figure 1: FAIRE isolation of open chromatin regions.
(a) Snapshot of open chromatin regions isolated from K562 cells using FAIRE. The fragment size is 200 bp. FAIRE peaks were called using MACS2 with default settings. **(b)** Distribution of 23,828 FAIRE-seq peaks in genomic features.



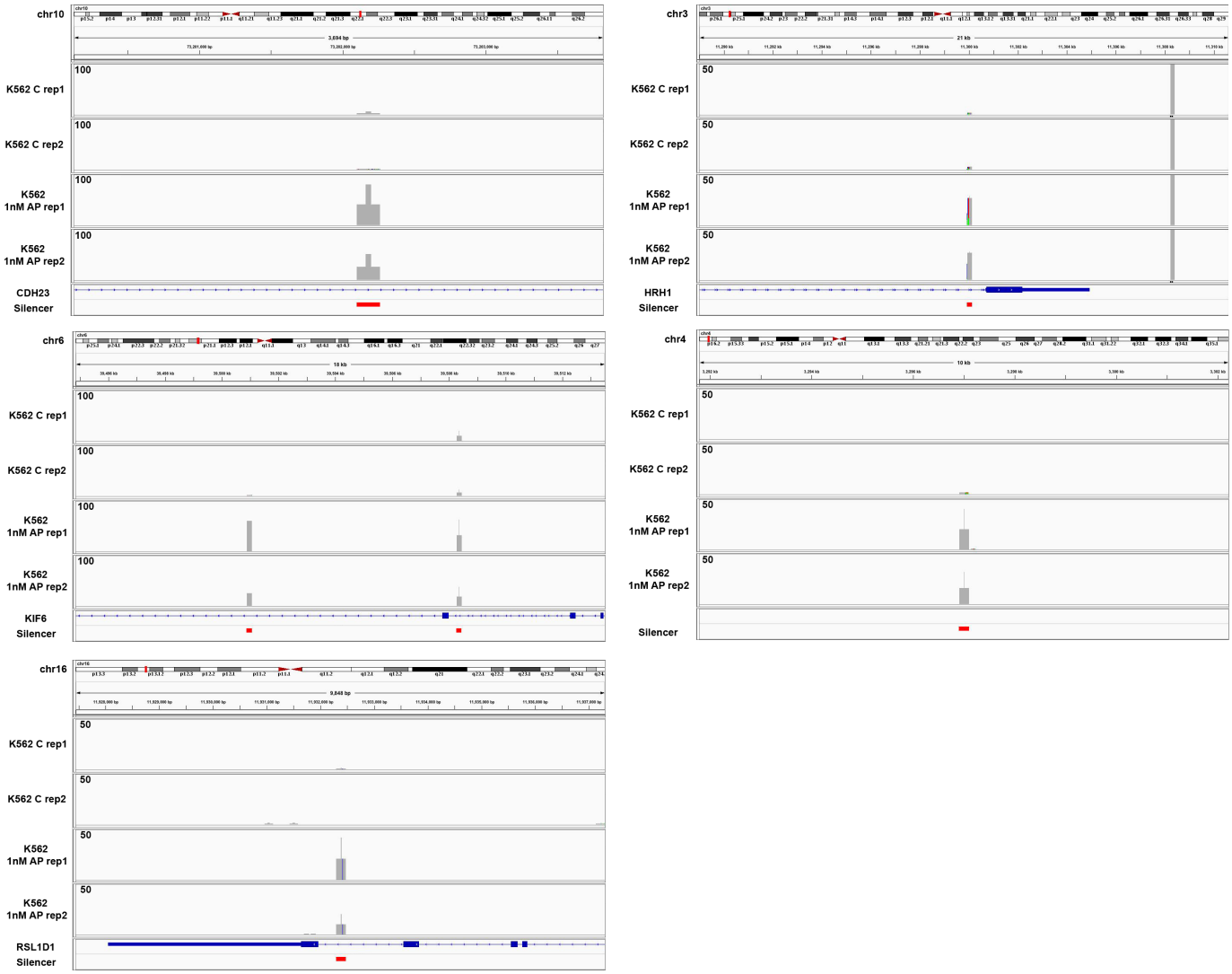
Supplementary Figure 2: Statistics of the tested library.
Distribution of all the tested fragments of the library in genomic features.



Supplementary Figure 3: Apoptosis induction in cells infected with the ReSE library.
Cells infected with the ReSE library with either 200 bp or 350 bp DNA fragments inserted were treated with dimerizer AP20187 (AP) at 0.1 nM or 1 nM to initiate apoptosis. PARP (Cell Signaling, #9542) cleavage is used as an indication of apoptosis. Actin (Thermo Fisher Scientific, #MA5-15452) is used as a loading control. These experiments were repeated twice independently with similar results. The blots were cropped. Full scans of the blots are shown in Source Data Full Gel Supplementary Figure 3.

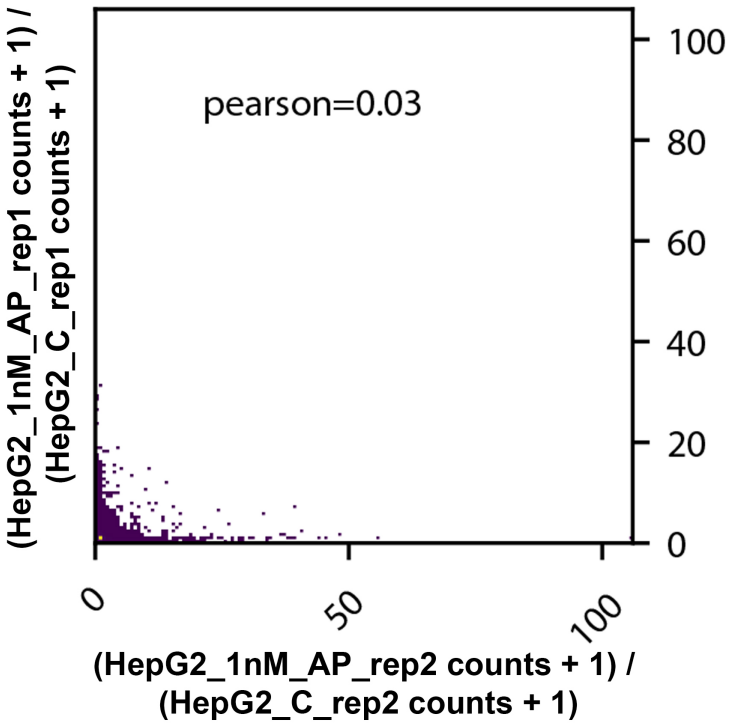


Supplementary Figure 4: Fold change comparison of the replicates from K562 cells.
Independent biological replicates from two ReSE screening in K562 cells were compared.



Supplementary Figure 5: Raw reads from two biological replicate experiments in K562 cells.
Raw read counts of silencer regions from untreated control replicates (upper two samples) and replicates after induction of apoptosis (lower two samples) are shown in the y-axis. Silencer regions are shown as red bars. Gene regions are shown when applicable.

a



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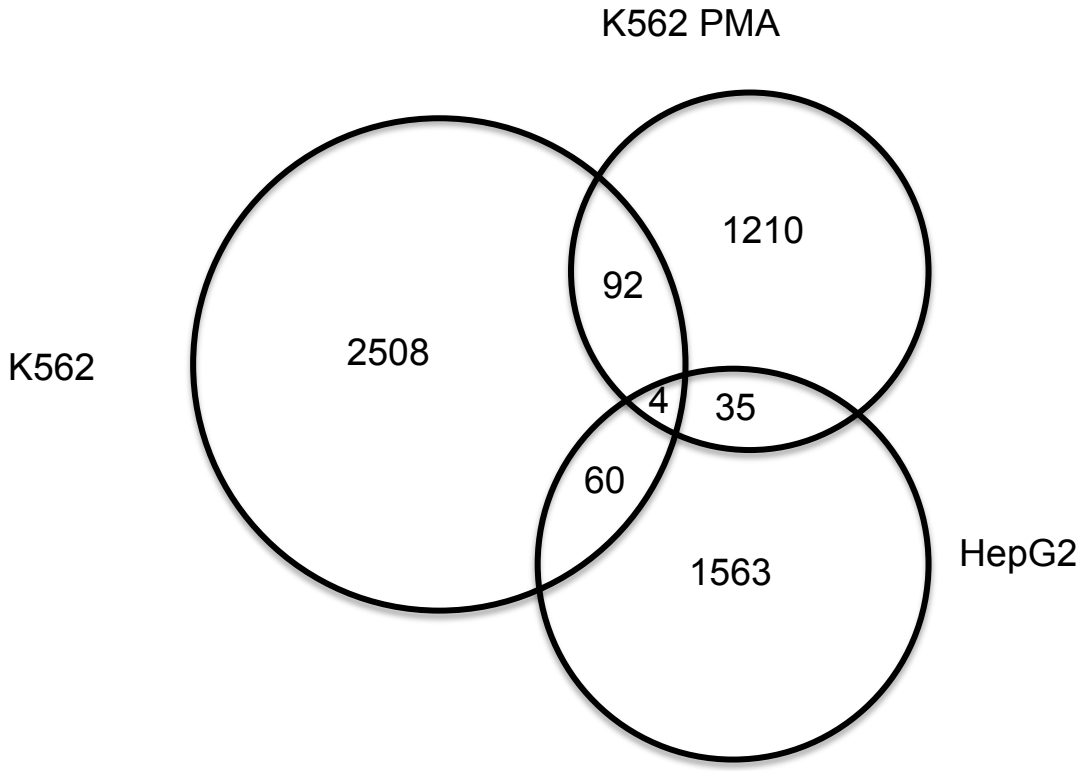
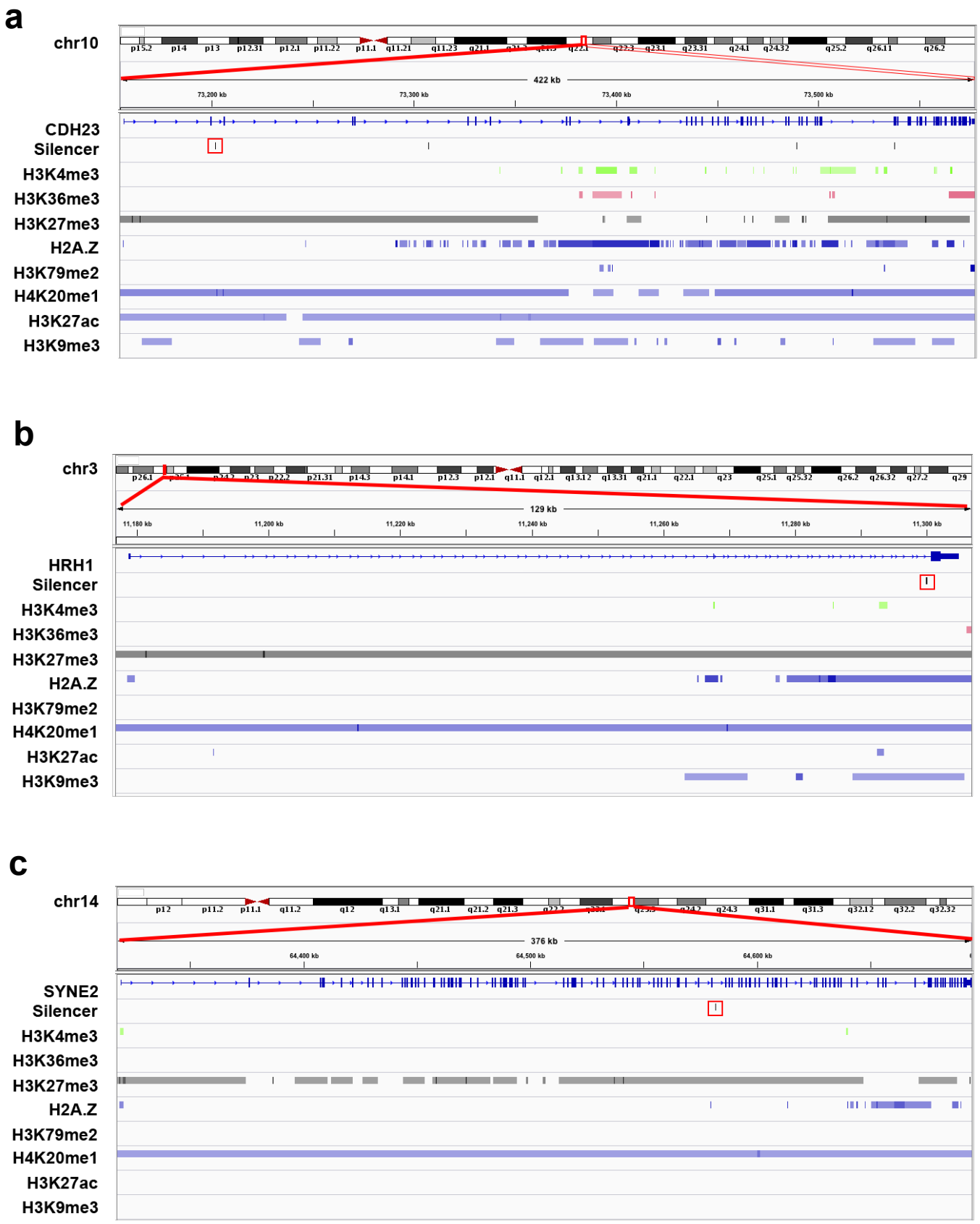
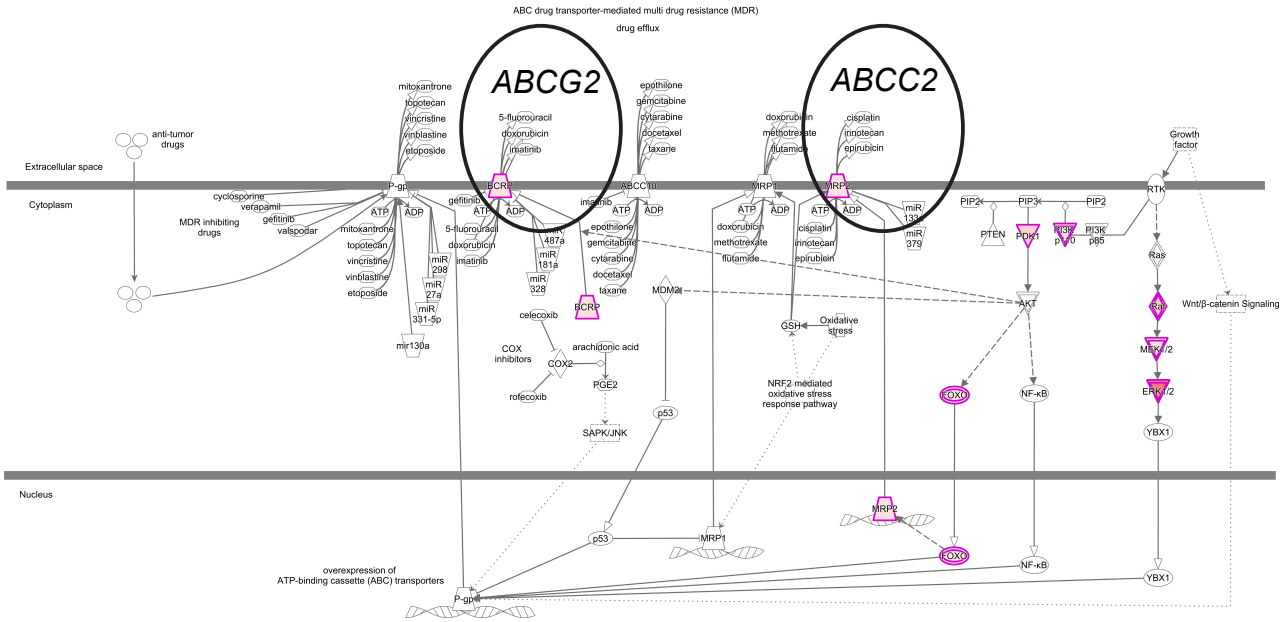


Supplementary Figure 6: ReSE screen results from HepG2 cells.
(a) Fold change comparison of the replicates. Independent biological replicates from two ReSE screening in HepG2 cells were compared. **(b)** Comparison of silencer regions identified from K562 cells, HepG2 cells and K562 cells differentiated by PMA. Overlapping was not random as determined by permutation tests (n = 20,000, adjust *P* value = 0.00005).

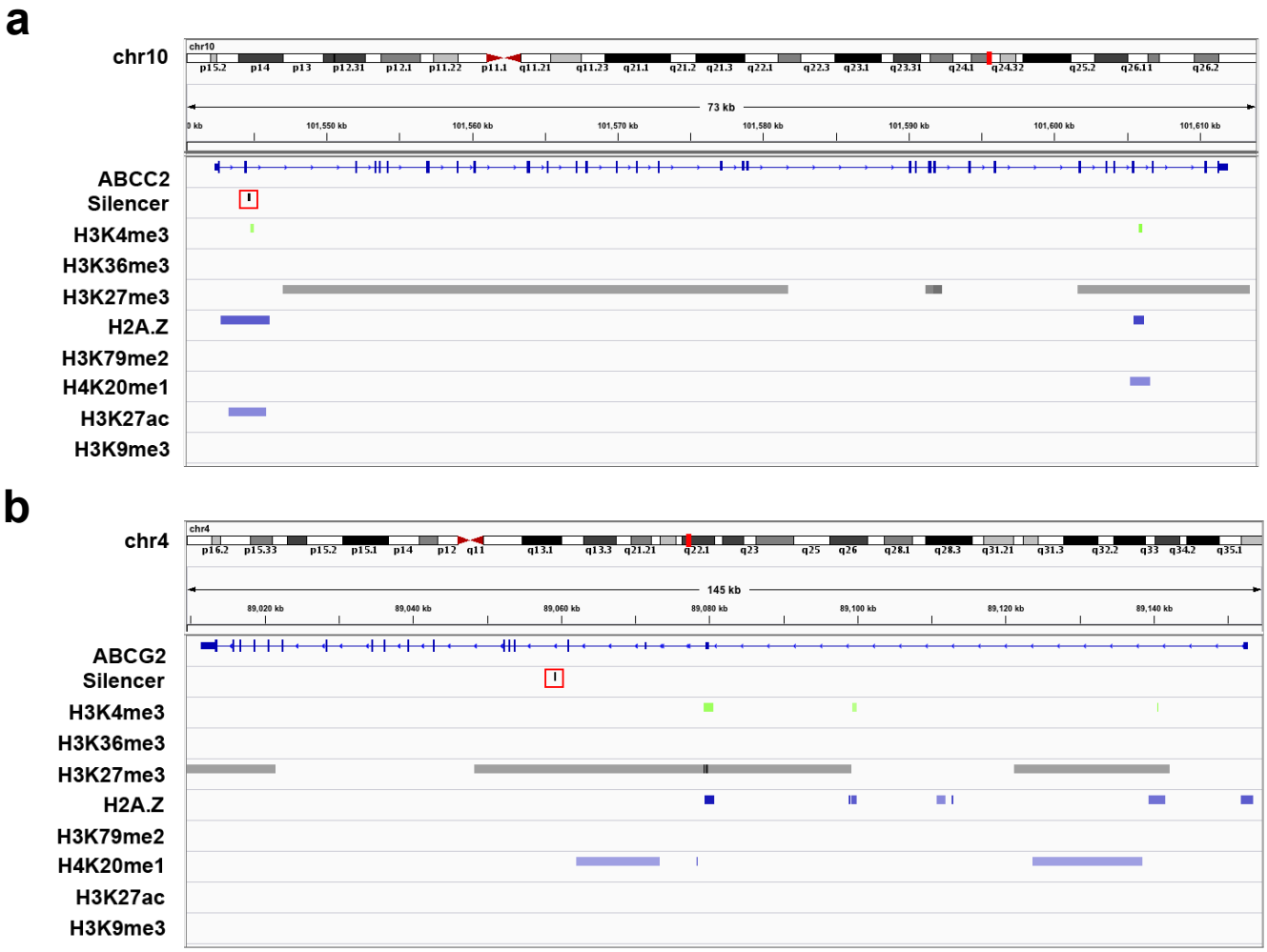


Supplementary Figure 7: Local histone modifications around the silencers from K562 cells.

Histone modifications of genes *CDH23* **(a)**, *HRH1* **(b)** and *SYNE2* **(c)** where silencer regions were tested in Extended Data Figure 3 are shown. Silencer regions that were validated using CRISPR deletion are highlighted in the red box.



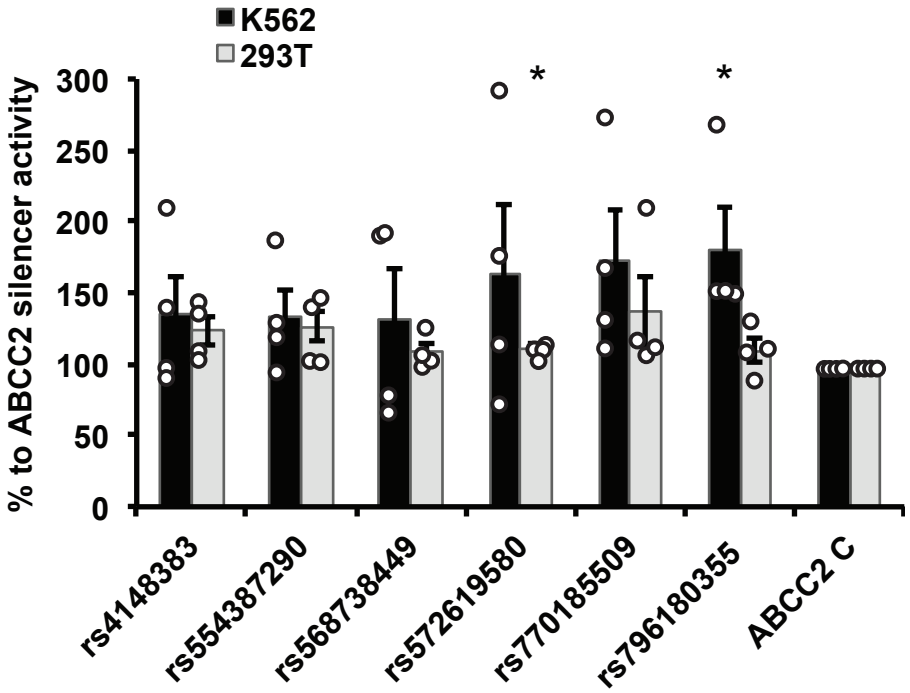
Supplementary Figure 8: Genes that harbor the ReSE silencers were enriched in the drug transporter pathway.
Genes containing the silencers were enriched in the ABC drug transporter pathway identified by the IPA analysis. -log(*P* value) = 2.671. *ABCC2* and *ABCG2* genes are highlighted.



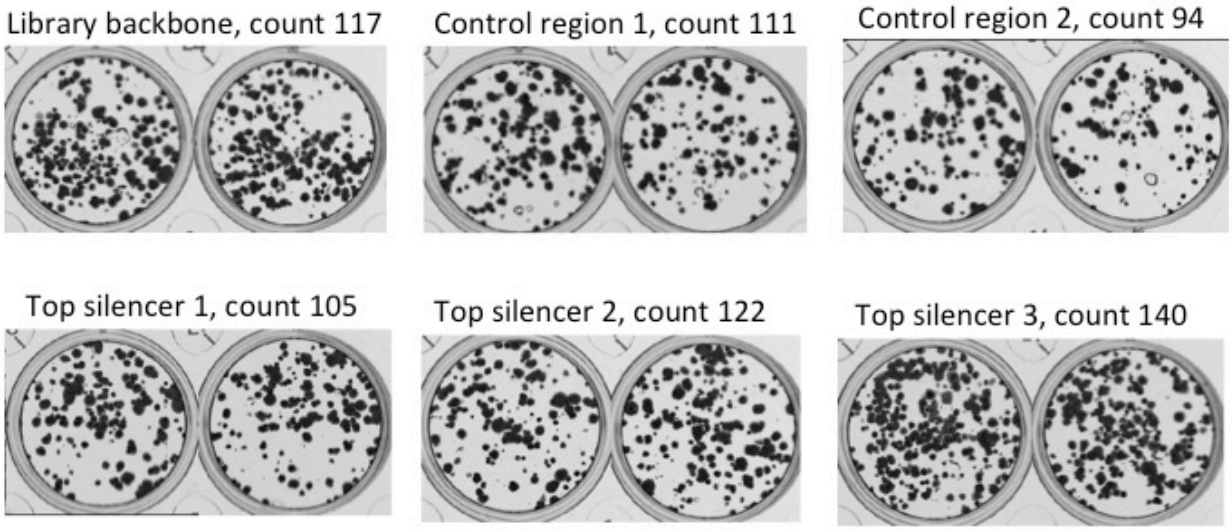
Supplementary Figure 9: Local histone modifications around silencers in K562 cells.
Histone modifications of *ABCC2* **(a)** and *ABCG2* **(b)** genes where silencer regions were tested in Figure 4 are shown. Silencer regions are highlighted in the red box.

a

b



Supplementary Figure 10: SNPs affect the repressive activity of silencers.
(a) SNPs in the silencer within the *ABCC2* gene were identified from dbSNP147. The respective SNP was introduced to the cloned *ABCC2* silencer region in the pGL4.53 luciferase reporter plasmid, using site-directed mutagenesis, and verified using Sanger sequencing. **(b)** Luciferase assays to determine the repressive activity of *ABCC2* silencer harboring different SNPs in K562 cells. The 293T cells were used as the control cell line to control the cell-type dependent repressive activity, and the original *ABCC2* silencer luciferase reporter plasmid was used as the control for baseline luciferase activity. The Y-axis represents the percentage of luciferase activity compared to that of the original *ABCC2* silencer in the respective cells (n = 4 biological independent samples; the bars show the mean value ± S.E.M; **P* value < 0.05, calculated using two-sided Student's *t* test). All exact *P* values are provided in Dataset 1.



Supplementary Figure 11: Silencers do not affect lentivirus packaging and puromycin selection in the ReSE assay.
Control or silencer fragments were amplified from the genomic DNA of K562 cells via PCR, and cloned individually into the screen library backbone plasmids. For all the tested plasmids, experiments were performed the same day under the same condition and protocol. Virus supernatant was collected and 293T cells were infected. Cells were fixed and stained after puromycin selection. Two replicates were included in the experiments, and the average counts were shown. No strong effects on virus production, infection and subsequent puromycin selection were observed when viruses were produced with the plasmids inserted with the silencer fragments, compared to that with the screen library backbone plasmids with no insertion or with random control DNA fragments.

Source Data Full Gel of Supplementary Figure 3